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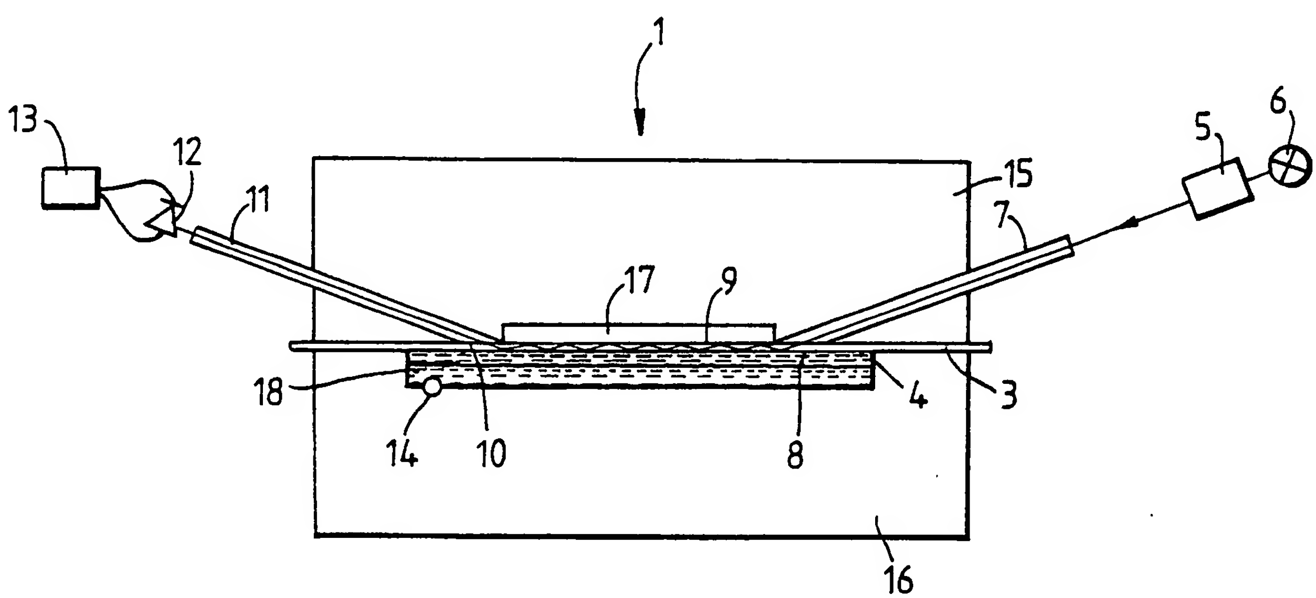

(57) Abstract

A method for determining the level of an analyte in a sample of whole blood uses the technique of internal reflection spectroscopy and an apparatus suitable for the technique. The method is of particular use to detect the attenuation of the totally reflected light by absorption of the evanescent wave, e.g., by haemoglobins. In the apparatus (1) incident light is directed via a light guide (7) to the reflection element (3) and the attenuated totally reflected light is directed out of the element to a photodetector (12) by a light guide (11). In the whole blood in the cuvette (18) the cells are removed from the interface (8) by gravity to leave blood cell free plasma (4) adjacent the interface (8). Thus analytes present in the plasma can be detected. The extent of haemolysis can be estimated.

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METHOD AND APPARATUS FOR DETERMINING THE LEVEL OF AN ANALYTE IN A SAMPLE OF WHOLE BLOOD

Background of the invention

The invention relates to a method for determining the level of an analyte in a sample of whole blood using internal reflection spectroscopy as the analysis technique.

Internal reflection spectroscopy is a well known analysis technique and has been the subject of a number of patent publications and other publications the contents of which are incorporated herein by reference. Pioneering work in the field was made by N.J. Harrick in the early '60ties and is disclosed i. a. in the book Internal Reflection Spectroscopy, Wiley 1967. A review has been given by P.A. Wilks, Jr. and Tomas Hirschfeld in the article: Internal Reflection Spectroscopy, Applied Spectroscopy Reviews, 1 (1), 99-130 (1967).

Briefly stated internal reflection spectroscopy is carried out by directing radiation with which to examine a sample so relative to a suitable reflection element as to be totally reflected one or several times at the internal surfaces of the reflection element before passing out of the element.

Total reflection occurs when incident light from an optically denser material of index of refraction n_1 is incident on an optically less dense material of index of refraction n_2 ($n_1 > n_2$) at an angle of incidence θ exceeding the critical angle θ_c obtained from the known laws of physical refraction:

$$\sin\theta_c = \frac{n_2}{n_1}$$

At the reflection site the incident light wave propagates into a narrow boundary layer in the less dense medium. If the less dense medium is absorbing, the propagating wave (the evanescent wave) is attenuated before its return. Alternatively, the evanescent wave may produce scattering or a fluorescence, phosphorescence or luminescence emission in the less dense medium.

A characteristic of the modified light, i.e., the attenuated radiation passing out of the reflection element, the scattered light or the fluorescence, phosphorescence or luminescence radiation, respectively, may be determined and utilised for analytical purposes to characterise the less dense medium.

The light wave propagating into the boundary layer is often referred to as the evanescent wave, the penetration depth of which is only a fraction of the wavelength of the incident wave. More specifically, the penetration depth, dp, as defined by the distance required for the electric field amplitude to fall to e^{-1} of its value at the surface is given by:

$$dp = \frac{\lambda}{n_1 \cdot 2\pi\left(\sin^2\theta - \left(\frac{n_2}{n_1}\right)^2\right)^{1/2}}$$

From the patent literature dealing with internal reflection spectroscopy the following patents can be mentioned as the most relevant patents known to the applicant:

US 3308709 (Harrick) which proposes a particular shape of an internal reflection element characteristic therein that a means for transmitting light into the reflection element and a means for extracting light from the reflection element have curved surfaces with the result that the number of reflections occurring and the depth of penetration of the beam into the rarer medium can be controlled;

US 3415602 (Harrick) which proposes another shape of an internal reflection element characteristic therein that the reflected beam be redirected from a point at a first surface through reflection at an opposed surface to the same point of the first surface, this making the element suited for use in connection with minute samples;

US 3431411 (Harrick) which proposes use of internal reflection spectroscopy in the analysis of aerosols;

US 3939350 (Kronick) which describes use of a reflection element in connection with an immunoassay method;

US 4040691 (David) and US 4106909 (David) which relate to the provision of a controlled humidity in the environment of a coated waveguide;

US 4050895 (Hardy) which proposes a waveguide having on its peripheral surface a material which selectively

combines with an analyte to measurably change the light transmitting properties of the waveguide;

US 4169676 (Kaiser) which relates to use of internal reflection spectroscopy for determining the amounts of metabolic products in blood. The patent claims a noninvasive method using a reflection element (an ATR-plate) placed against biological tissue but mentions also briefly that tests were performed in which blood removed from to body was allowed to run over the measurement surface of the reflection element. According to the disclosure of this patent the determination is performed by means of infrared light and there is no mention either of any influence from the blood cells or of any special orientation or configuration of the reflection element needed to make the element suitable for whole blood use;

US 4321057 (Buckles) corresponding to WO 8100912 which discloses an analytical device comprising an longitudinal waveguide. A preferred embodiment suitable for use in connection with whole blood is provided with a barrier layer excluding large molecules and blood cells;

US 4368047 (Andrade) and US 4447546 (Hirschfeld) which relate to use of internal reflection spectroscopy in connection with immunoassay;

DE 2837769 and DE 2928419 (Müller) which deal with the problems of obtaining optically clean surfaces of multi use reflection elements when applying these to biological media;

EP 75353 (Battelle) which relates to application of internal reflection spectroscopy to an analytical process where an analyte reacts with a specific reactant resulting in the formation of an analyte-reactant product on the surface of the reflection element.

EP 170376 (Unilever) which relate to an optical analysis method utilizing only a particular part of the light emerging from a reflection element for the measurement.

Kaiser in IEEE Transactions on Biomedical Engineering vol BME-26 pp 597 to 600, describes the use of lasers as the light source in internal reflection spectroscopy using infra-red spectroscopy on citrate blood. IR spectroscopy is of limited use for biological samples because of the interference of water molecules which complicates interpretation and because only a limited range of components can be detected at those wavelengths. Furthermore citrate ions complex with cations, especially calcium ions which means that the concentration of those ions in the sample cannot be determined. The blood is said to run over the wave guide.

Gendreau in Applied Spectroscopy (1987) 35 353-357 describes the use of infra-red spectroscopy with attenuated total reflection techniques to study the adsorption on to a surface of proteins from whole blood. The use of IR spectroscopy has the problems and limitations mentioned above. The waveguide appears to be surrounded by the liquid sample.

EP 185126 (Battelle) describes the use of internal reflection spectroscopy in which the light totally reflected at each of two or more surfaces of waveguides in contact with a sample is analysed for simultaneous measurement of two or more parameters. In one example one surface is coated with a reactant which specifically reacts with an analyte in the sample and the other surface is uncoated (or is coated with a component to prevent absorption of compounds in the sample), and the transmitted light from the respective waveguides compared. In practice all the experiments described would be carried out on haemolysed blood to obtain the desired measurements. The apparatus illustrated uses the vertical sides of the sample cuvette as the waveguides.

DE 3532563 (AVL AG) describes apparatus in which a waveguide carries a coating which is in contact with the sample. The fluorescence of a component in the coating depends on the concentration of an analyte in the sample. Light may be conducted to the coating by total reflection in the uncoated part of the waveguide and refraction into the coating which has a refractive index intermediate that of the waveguide and the sample. The fluorescence radiation emitted is transmitted directly to detection means. The exciting radiation transmitted is not analysed, nor is the attenuation of the radiation by absorption of the evanescent wave detected.

The following description of currently practiced optical methods for the analysis of blood samples is relevant to the background of the invention. All such methods utilize various provisions so as to produce an optically clear sample. Among these various provisions should be mentioned pretreatment of the blood sample so as to separate it into a plasma or serum phase and blood cells before introducing the sample into the analysis apparatus; the use of disposable elements provided with a filter layer which retains the blood cells and allows the plasma phase pass through the filter layer; incorporation of separation means such as dialysis, centrifugation or hemolysis in the analysis apparatus.

In clinical analysis it is a constant and strongly emphazised objective to make the operation of the procedures/analyzers as simple and fast as possible in order that the results provided are maximally relevant to patient care. The ability to perform analyses in proximity to the patient and outside of a central laboratory is an advantage. Furthermore, the economic cost of analysis is at present a major consideration.

In accordance herewith the object of the present invention is to provide an improved analysis method for use on whole blood.

Disclosure of the invention

The method according to the present invention for determining the level of an analyte in a sample of whole blood using internal reflection spectroscopy as the analysis technique and with a reflection element exposed to the sample is characterized by orienting the reflection element so relative to the sample and the gravity field or a superimposed force field that the blood cells under the influence of the said field are removed from one or several reflection sites, the active reflection sites, of the reflection element, and performing the determination after a boundary layer comprising essentially blood cell free plasma phase has been provided contiguous to the said active reflection sites.

The method according to the present invention provides a highly advantageous and easily implementable optical method of analyzing whole blood eliminating the need for pretreating the blood sample in order to make it optically clear. In its most straightforward embodiment the field of gravity is utilized to draw the blood cells away from the active reflection sites of the reflection element leaving a boundary layer of blood cell free plasma phase contiguous to the active sites of the reflection element. The removal of the cells

from the reflection site(s) may be accelerated by addition of an additive, such as an anti-coagulant, preferably one which does not form complexes with cations, more preferably heparin.

This boundary layer will be established within a very short period. If heparin is used as an anticoagulant, aggregation of blood cells occurs resulting in their more rapid migration under gravity and accelerating the formation of the boundary layer. We have found that from a resting heparinised blood sample a boundary layer of adequate thickness ($\sim 1\mu m$) will be formed within a fraction of a second. Thus, the separation provided for according to the method of the invention does not to any significant extent add to the time per analysis.

When utilizing the field of gravity in the formation of the blood cell free layer no additional means of achieving separation which increases the cost of the procedure in terms of time or of economics, is necessary. However, it may for some purposes be advantageous to subject the blood cells to a superimposed force field such as e.g. a centrifugal field or any other field which is able to move the blood cells in order to achieve the blood cell free plasma phase.

The reflection element may have any suitable shape such as a plate, a prism or a fiber and may be produced from any material of adequate optical properties. Glass, quartz as well as plastics material may be used for the reflection element.

Preferably, the waveguide comprising the reflection element is made very thin; a thickness less than 100 µm, preferably 10-50 µm is advantageous. The reason is that for a given length of the reflection element the number of reflection sites is greater for a thinner reflection element than for a thicker reflection element. The increased sample interaction seen for thin reflection elements results in greater sensitivity.

In order to provide adequate mechanical properties it may be advantageous to provide the reflection element as a coating on a substrate. In that case the substrate should be made from or comprise a material giving rise to total reflection at the interface between the reflection element and the substrate.

Within a measurement sequence those reflection sites on the reflection element which do not contact the blood cell free layer should be subjected to surroundings of standard optical conditions such as constant refractivity index and absorptivity.

It is contemplated that the method according to the invention may be used for the determination of a great number of species present in blood. Among those species should be mentioned hemoglobins, bilirubin, total protein, glucose and creatinine. A suitable wavelength or wavelength range for the performance of the method is chosen from the spectral properties of

the species to be detected. It is highly preferred to use UV or visible light, since IR light is absorbed by water molecules which renders the analysis of the transmitted light complex.

For the determination of glucose and other metabolites it is envisaged that a wavelength from the IR range, preferably the near IR range, would be advantageous. For the determination of bilirubin and hemoglobins a wavelength selected from the visible or near IR range is assumed to be appropriate and for the determination of total protein a wavelength from the UV range should be selected. The method is of particular value in the measurement of bilirubin, and even more, preferably of haemoglobin in the plasma. By this method the extent of haemolysis can be estimated.

Analytes which are not directly detectable may be determined after exposure of the sample to a suitable reagent and formation of a reaction product between the analyte and the reagent which reaction product may

then be determined according the method of the present invention. The reagent may be added to the sample or may be immobilized as a thin, preferably monomolecular coating, at the blood contacting surface of the reflection element. Illustrative examples of such analytes/reagents are H^+/pH indicators; O_2/redox indicators.

A considerable number of analytes may be measured enzymically by coupling analyte-specific enzyme reactions either directly or via further enzyme reactions to the formation or consumption of reduced nicotinamide adenine dinucleotide or reduced nicotinamide dinucleotide phosphate. Such reactions may be measured by means of this invention in whole blood by measurement of absorbance of the evanescent wave at 340 nm or 366 nm, or by measurement of fluorescence due to the evanescent wave. Illustrative examples of such analytes/enzyme are: glucose/hexokinase, glucose-6-phosphate dehydrogenase; urate/uricase, xanthine oxidase.

Other analytes may be measured enzymically in whole blood by measuring O_2 or H^+ generation or consumption, directly or via intermediate reactions, by observing changes in pH indicators or in redox dyes by means of this invention. Illustrative examples of such analytes/enzymes are: amino acids/amino acid decarboxylases; glucose/glucose oxidase.

Furthermore, analytes may be measured in whole blood by enzyme-linked or by other non-isotopic immunochemical techniques which may be observed by optical means by the method of this invention.

It is noted that an essential element of all of the given examples is that neither the reagents nor the reaction products cause damage to the cellular elements present in the blood sample. This method may be used in conjunction with a separate measurement step in which hemolysis or cytolysis is caused for the purpose of including the contents of the cells in the measurement.

In a preferred embodiment of the invention, the method includes a step of analysing the said sample by a different technique, suitably using an electrochemical measuring electrode, for instance an ion selective electrode, e.g., K^+, Na^+ or Ca^+ ion selective electrode, a pH electrode or an oxygen electrode. Preferably the method is used to estimate the extent of hemolysis in the sample, and this allows the values obtained by the analysis by the electrode to be corrected to give the plasma levels of the species observed.

The invention also relates to an apparatus for determining the level of an analyte in a sample of whole blood, said apparatus comprising a reflection element having an entrance part for receiving light transmitted to the reflection element and an exit part for said light having passed the reflection element, said apparatus being characterised by an active front part having a sample contact surface with one or several reflection sites, said reflection element being so located relative to the sample in the normal use of

the apparatus that the blood cells under the influence of the gravity field or a superimposed force field be removed from the active front part of the reflection element.

The light is totally internally reflected in the reflection element between the entrance part and the exit part, and is attenuated in use by the sample. The exit part is, for instance, suitable for connection to a detector for analysing the attenuation of the totally internally reflected incident light.

It is understood that in the apparatus according to the invention any reflection sites located out of contact with the sample should be subjected to surroundings of standard optical conditions.

In a preferred embodiment utilizing the gravity field to remove the blood cells from the active front part of the reflection element the apparatus according to the invention comprises sample inlet means and sample cuvette means connected to the sample inlet means, the reflection element being located so that its sample contact surface provides an upper wall of the sample cuvette.

In a further preferred embodiment of the apparatus according to the invention a light source means for transmitting light to the reflection element and a light detection means for receiving light transmitted from the exit part of the reflection element have been provided.

The light source means and the light detection means may be any convenient means well known _per se_. They are preferably suitable for emitting and detecting, respectively, ultra violet or visible light. The light source may emit monochromatic light or polychromatic light. In the latter case means for selecting the wavelength useable in the performance of the method according to the invention may be provided in the light path either upstream to the entrance part of the reflection element or downstream to the exit part of the reflection element but upstream to the light detection means.

A suitable light detector means is a photoelectric device producing a current or voltage signal reflecting the intensity of the light received. The apparatus according to the invention preferably contains, or is connected to, suitable signal processing means and output means which are well known _per se_.

For some purposes it would appear appropriate to provide the apparatus according to the invention as a disposable unit. In that case the light source means and the light detector means are preferably located in a device adapted to receive the disposable unit and

the apparatus according to the invention is characterized by comprising light inlet means for optically coupling the apparatus to external light

source means and light exit means for optically coupling the apparatus to external light detection means.

In order to make the apparatus suited for multiple analysis on the same sample of blood it is preferred to include further measuring means, e.g. an electrochemical measuring electrode, in the apparatus.

However, the apparatus according to the invention may also be embodied as a device suited for insertion in a sample contained in a test tube or the like.

Such device according to the invention for determining the level of an analyte in a sample of whole blood accomodated in sample container means is characterized by comprising a shield enclosing a substantial part of the device but leaving exposed a sample contact surface of an internal reflection element; light guide means for directing light into and away from the reflection element; means for attaching the device to the sample container means in order to maintain the device quiet relative to the sample during a measurement period and for orienting the sample contact surface of the reflection element so that the blood cells under the influence of the gravity field or a superimposed force field be removed from the sample contact surface.

The invention also relates to an analyzer for the determination of the level of potassium in a sample of whole blood, and comprising potassium measuring means; said analyzer further comprising means for detecting the level of hemolysis in the said sample.

In connection with measurement of the level of potassium in whole blood it is of major importance that the sample has not undergone even a slight degree of hemolysis, i.e. rupture of the blood cells. This is because blood cells contain a far higher potassium level than the plasma phase and hence even a slight hemolysis of a few percent may give rise to serious error in potassium determinations.

Thus, for example, if 3% hemolysis has occurred in a sample of blood with a normal cell count, plasma potassium concentration is increased by about 2 mmol/L. This could result in a patient with life-threatning hypokalaemia being adjudged to be normal and being thereby denied appropriate treatment, or in a patient with normal plasma potassium concentration being adjudged to be hyperkalaemic and thereby treated inappropriately.

This means that a potassium analyzer provided with means for detecting the level of hemolysis in the blood sample will enable the physicians to evaluate the clinical reliability of potassium values.

It is preferred that the means for detecting the level of hemolysis in the blood sample comprises a reflection element. A preferred potassium analyzer according to the present invention is characterized by comprising sample inlet means and sample cuvette means connected to the sample inlet means for accomodating the sample and exposing the sample to the reflection element the reflection element being provided in an upper wall of the sample cuvette means.

In this connection should be mentioned that "accomodating the sample" should mean accomodating the entire sample volume or only part thereof.

Brief description of the drawings

The invention is further illustrated by way of example by the following description and accompanying drawing, wherein

FIG. 1 illustrates an experimental set-up for performing the method according to the invention:

FIG.2 is a graph showing the results obtained by the experimental set-up of FIG. 1;

FIG.3 shows diagrammatically a whole blood potassium analyzer provided with a reflection element for detecting the level of hemolyzation;

FIG. 4 shows diagrammatically a whole blood analyzer provided with means for adding a reagent to the sample;

FIG. 5 is a longitudinal section through a cuvette of an automated analyzer;

FIG. 6 is a longitudinal section through a similar disposable cuvette;

FIG. 7 shows a device suitable for insertion in a test tube;

FIG. 8 shows in enlarged fragmentary longitudinal section, the active front part of the device of FIG. 7.

<u>Preferred embodiments of the invention</u>

FIG.1 illustrates diagrammatically an experimental set-up used in the evaluation of the method according to the present invention.

A block-shaped container means 1 comprises two half parts 15 and 16 having a 0.15 mm thick glass plate 3 sandwiched between them. Each half part is made from a 10 x 10 x 30 mm^3 block of a black coloured polyoxymethylene material sold under the trade name DELRIN. The glass plate 3 is a 0.15 mm thick microscope glass cover slip manufactured by Chance Propper Ltd., England. The glass plate is fixed to each of the two half parts 15 and 16 of container means 1 by means of an optically clear silicone adhesive.

Centrally in the upper half part 15 a recess 17 of length about 14 mm, width about 9 mm and depth about 1 mm has been provided. Centrally in the lower half part 16 a recess 18 of length about 20 mm, width about 4 mm and depth about 2 mm has been provided forming a cuvette for accomodating a blood sample.

In the upper half part 15 adjacent to recess 17 is provided light guide means 7 and 11. Light guide means 7 and 11 are light guide monofilaments sold under the designation Crofon OE-1040 by Du Pont de Nemours. Prior to mounting in half part 15 the shielding material has been removed from light guide means 7 and 11.

The blood sample is introduced by means of a syringe into recess of cuvette 18 through a capillary bore (not shown) of diameter 1.1 mm provided in the lower half part 16. The capillary bore extends perpendicularly to the plane of FIG. 1 and opens into the front wall (not shown) of cuvette 18 adjacent to the right side wall of and the bottom wall of cuvette 18. Surplus sample is discharged through capillary bore 14. Due to the gravity field a blood cell free plasma phase 4 is formed in cuvette 18 contiguous to glass plate 3.

By means of a monochromator 5 (H20 VIS, Jobin Yvon, France) light of two alternate wavelengths 506 nm and 548 nm is extracted from the light emitted from a 20 W halogen lamp 6.

The monochromatic light beam emerging from monochromator 5 is guided to glass plate 3 through plastics waveguide 7. The refractive indices of plastics waveguide 7 and glass plate 3 and the angle between the longitudinal axis of waveguide 7 and glass plate 3 are so chosen that the light traverses the interface between waveguide 7 and glass plate 3 without being reflected at that interface. The actual angle from the glass plate to the longitudinal axis of the waveguide is 20^{o} (counter clockwise). When the light reaches the interface 8 between glass plate 3 and blood cell free plasma phase 4 it is totally reflected whereupon the light traverses the glass plate to the upper interface 9 between glass plate 3 and air. At the interface 9 the light is reflected whereupon the light traverses glass plate 3 to interface 8 etc. Thus, glass plate 3 acts as a reflection element. After having traversed glass

plate 3 a number of times the light reaches interface 10 between glass plate 3 and plastics waveguide 11. At that point the light emerges from glass plate 3 and is transmitted through waveguide 11 to a photodector 12 (BPX 90 photodiode, Siemens). The current signal of photodetector 12 is proportional to the intensity of the light reaching photodetector 12 and is measured by means of a galvanometer 13 suited for measuring current intensities from the pA range (GVM30, Radiometer A/S).

At the reflection sites on interface 8 between glass plate 3 and blood cell free plasma phase 4 an evanescent wave emerges. If plasma phase 4 contains any light absorbing species these will modify the intensity of light reaching the photodetector 12. This means that e.g. the presence of reduced hemoglobin (Hb) and/or oxyhemoglobin (HbO_2) in plasma phase 4 will cause attenuation of the light reaching photodetector 12 reflecting the level of reduced hemoglobin and/or oxyhemoglobin in plasma phase 4, if the light intensity measurement at photodetector 12 is effected for a suitable wavelength. In this connection it should be mentioned that the presence of reduced hemoglobin and/or oxyhemoglobin in plasma phase 4 indicates that hemolysis has taken place in the blood sample. Either of the above-mentioned wavelengths of 506 nm and 548 nm are isobestic as to Hb and HbO_2, i.e. the molar absorptivity of Hb and HbO_2 at either one of these wavelengths is the same (but different from the molar absorptivity at the other wavelength).

In order to determine the relationship between degree of hemolysis of a sample of whole blood and the current signal of photodetector 12 a series of measurements was made with the experimental set-up of FIG. 1. As basis material was used a heparinised blood sample containing 13.4 g% (8.32 mM) Hb + HbO_2 as measured on an oximeter of the type OSM2 manufactured and sold by Radiometer A/S, Copenhagen, Denmark.

The blood sample was divided into two fractions one of which was hemolyzed by freezing at -20 ^{o}C. Unhemolyzed sample material and hemolyzed sample material was mixed in the ratios:

100:0; 80:20; 60:40; 20:60; 20:80; 0:100.

in order to provide samples for the above mentioned measurement sequence.

After completion of the measurements on the above 6 samples the unhemolyzed sample fraction was subjected to centrifugation in order to obtain a reference standard of pure plasma phase and measurement was made on this reference standard, too.

Assuming that the modification of the light caused by plasma phase 4 obeys Lambert Beers Law:

$$\log \frac{I}{I_o} \propto C$$

wherein I is the intensity of light after interaction with a sample; I_o is the intensity of light of interaction with the reference standard and C is the

concentration of the analyte ($Hb + HbO_2$) in the sample, a linear relationship between log I/I_o and C should be found.

A linear relationship should also be found between

$$\log \frac{(I/I_o)_{\lambda 1}}{(I/I_o)_{\lambda 2}} = \log \frac{I_{\lambda 1}/I_{\lambda 2}}{I_{o\lambda 1}/I_{o\lambda 2}}$$

and C.

λ_1 and λ_2 are the actual wavelengths.

The results obtained in the measurement sequence described are given in table 1 below:

	Degree of hemolysis					
	0 %	20 %	40 %	60 %	80 %	100%
$\log \frac{I_{\lambda 1}/I_{\lambda 2}}{I_{o\lambda 1}/I_{o\lambda 2}}$	-0.006	-0.061	-0.088	-0.102	-0.147	-0.14

Table 1

The results given in table 1 are shown on FIG. 2 in which

$$\log \frac{I_{\lambda 1}/I_{\lambda 2}}{I_{o\lambda 1}/I_{o\lambda 2}}$$

indicated as log I/I_o is plotted against degree of hemolysis.

An experiment was made to evaluate the usefulness of a set-up in which the upper surface of the reflection element constitutes the sample contact surface.

In that experiment container means 1 with glass plate 3 was turned upside-down so that the reflection element was beneath the sample. The sample was unhemolyzed whole blood. A signal was obtained from light detector 12 corresponding to the signal representing 100% hemolysis in the set-up embodying the present invention. This is ascribed to the effect of the blood cells. Thus, the necessity of the relative orientation between reflection element and whole blood sample provided for according to the present invention is demonstrated.

FIG. 3 shows diagrammatically an analyzer 21 according to the invention for analyzing samples of whole blood. From a sampling device, in the present case a syringe 22, a sample is introduced in the analyzer 1 through sample inlet means 23. The sample is drawn through a sample conduit 24 of analyzer 1 by means of pump means shown as 25. When the sample reaches particular measuring locations or cuvettes 26 and 27 in conduit 24 the sample transport is stopped. In cuvette 26 the sample is contacted with a potassium measuring electrode 28 and a reference electrode 29. In cuvette 27 a reflection element 30 forms part of the upper wall of cuvette 27, reflection element 30 further being connected to light source means 31 and light detector means 32 via light guides 33, 34, respectively. Light guides 33, 34 are so oriented relative to reflection element 30 that no reflection takes place at interfaces 35, 36 between light guides 33, 34 and reflection element 30. At the reflection sites contacting the sample a modification of the light intensity takes place if any light absorbing compounds are present in the sample boundary layer

contiguous to the sample contact surface of reflection element 30. The reflection sites which are out of contact with the sample are subjected to surroundings, the optical properties of which remain constant ("standard optical properties") during at least one measurement sequence including measurement on a sample as well as on a reference standard and are shielded against stray light.

By means of reflection element 30 and appended light source means 31 and light detector means 32 the total concentration of hemoglobins ($Hb + HbO_2$) is determined utilizing light of appropriate wavelengths. It is to be understood that between light source means 31 and reflection element 30 as well as between reflection element 30 and light detector means 32 appropriate optical equipment may be provided, such as monochromator means, lenses etc..

The signals from the electrochemical measuring system comprising electrodes 28 and 29 and from light detector means 32 are fed to suitable data processing means 37 and output means 38 via A/D converting means 39. Means 37, 38, 39 are considered as well known _per se_ in connection with analyzers of the present nature and will not be further described in the present context.

After completion of the measurement procedure in cuvette 27 the sample is transported via sample conduit 24 to the outlet 40 of analyzer 21 and discharged into waste container 41.

As is well-known in the art the apparatus 1 is intermittently calibrated on a calibration or reference standard to which values for the analyte(s) in question have been assigned.

FIG. 4 shows diagrammatically an analyzer 111 for determining the level of an analyte in a sample of whole blood. From a syringe 112 a sample is introduced through sample inlet means 113 and drawn through a sample conduit 114 of analyzer 111 by means of pump means 115.

Reagent forming with the analyte a light absorbing reagent/analyte complex or a complex otherwise modifying the light transmittance is pumped from reagent reservoir 116 by means of pump means 117 through a reagent conduit 118 into sample conduit 114. The sample/reagent mixture is further transported to a particular measuring location or cuvette 119 in sample conduit 114. When the sample/reagent mixture reaches the cuvette 119 the transport of the mixture is stopped. In cuvette 119 a reflection element 129 forms part of the upper wall the reflection element being connected to light source means 120 emitting light of a relevant wavelength and light detector means 121 via light guides 122, 123, respectively. The cuvette and appended optical devices are constructed as explained above in connection with FIG. 3.

By means of structure 119, 120, 121, 122, 123, 129 and the appended conventional electronic signal processing and output means (A/D converting means 124; data processing means 125 and display/printer means 126)

the level of analyte/reagent complex is determined and correlated to the level of the analyte originally present in the sample.

After completion of the measurement the sample/reagent mixture is transported via sample conduit 114 to the outlet 127 of analyzer and discharged into waste container 128.

FIG.5 shows in more detail a cuvette generally designated 100 for use in an automated analyzer and corresponding to cuvettes 7, 119 of FIG. 3 and 4.

Sample enters into cuvette 100 through sample inlet 101 and accomodates space 102 of cuvette 100. A reflection element 103 made from an optically clear material provides the upper wall of cuvette 100. In order to facilitate entrance and exit of light and at the same time provide an appropriate sensitivity the reflection element 103 has been given the configuration shown with enlarged end parts and a thin sample contact part. The reflection element 103 is supported on substrate 104 made from a material of lower refractivity index than reflection element 103. The thickness of reflection element 103 is preferably less than 1 mm, preferably less than 100 um and particularly 10-50 um. Light enters reflection element 103 through light guide 105 and emerges from reflection element 103 through light guide 106.

After completion of the measurement procedure sample is discharged from cuvette 100 through sample outlet 107.

FIG. 6 shows another embodiment of the apparatus according to the invention.

A disposable cuvette unit 200 is adapted to fit into a permanent part 201. The two parts 200 and 201 are interlocked by means of snap lock 202. Sample is introduced into the space 203 of cuvette unit 201 so as to contact an upper reflection element 204 supported on a substrate 205.

In the permanent part 201 light guide means 206 and 207 are provided. Light guide means 206 guides light from external light source means (not shown) to light inlet means 208 of reflection element 204 and light guide means 207 guides light from light exit means 209 of reflection element 204 to external light detector means (not shown). Thus, light inlet means 208 and light exit means 209 provides for optical coupling of the apparatus according to the invention, i.e the disposable unit, to the permanent part 201 with the appended light source and light detector means.

FIG. 7 shows a further device 300 useful in the performance of the method according to the present invention. The device 300 is an elongate member having an active front part comprising a reflection element 301 and a shielding 302 covering the peripheral edge of reflection element 301 as well as the body part 303 of device 300. At the rear end of device 300 is provided collar means 304 for mounting device 300 to a sample container, e.g. a test tube. Instead of collar means 304 other suitable means could be used for attaching the device to a sample container in order to keep device 300 quiet relative to the

sample during a measurement sequence. Finally, cable 305 connecting device 300 with external light quide and light detection means is shown. Device 300 may be held in any upwardly oriented direction.

The front part of device 300 is further illustrated in FIG. 8 wherein like reference numerals designate like parts. In the embodiment shown reflection element 301 has been rearwardly extended providing longitudinally extending light guide means 306 and 307. Reflection element 301 and light guide means 306 and 307 are supported on substrate 308 of lower refractivity index. The bevelled edges 309 and 310 of the reflection element direct the light into and away from the sample contact part of the reflection element, respectively. Enclosed between the bevelled edges 309 and 310 and sheath 302 are material 311 and 312 of lower refractivity index than reflection element 301, e.g. air.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

CLAIMS

1. A method for determining the level of an analyte in a sample of whole blood using internal reflection spectroscopy as the analysis technique and with a reflection element exposed to the sample, said method being characterized by,

orienting the reflection element so relative to the sample and the gravity field or a superimposed force field that the blood cells under the influence of the said field be removed from one or several reflection sites, the active reflection sites, of the reflection element,

and

performing the determination after a boundary layer comprising essentially blood cell free plasma phase has been provided contiguous to the said active reflection sites.

2. A method according to claim 1, characterized in that the analyte is a species absorbing visible light.

3. A method according to claim 1 or 2, characterized in that the analyte is selected among hemoglobins and bilirubin.

4. A method according to claim 1, characterized in that the analyte is a species absorbing UV light.

5. A method according to claim 4, characterized in that the analyte is total protein.

6. A method according to claim 1, characterized in that the analyte is a species absorbing IR light.

7. A method according to claim 7, characterized in that the analyte is a metabolite.

8. A method according to claim 8, characterized in that the analyte is glucose.

9. A method according to claim 1, characterized by exposing the sample to an agent reacting with the analyte and determining the level of the analyte in the sample via a determination of the reaction product thus formed.

10. An apparatus for determining the level of an analyte in a sample of whole blood, said apparatus comprising,

a reflection element having an entrance part for receiving light transmitted to the reflection element and an exit part for light having passed the reflection element;

said apparatus being characterized in that the reflection element is provided with an active front part having a sample contact surface with one or several reflection sites, said reflection element being so located relative to the sample in the normal use of the apparatus that the blood cells under the influence of the gravity field or a superimposed force field be removed from the active front part of the reflection element.

11. An apparatus according to claim 10, characterized in that it further comprises,

sample inlet means;

sample cuvette means connected to the sample inlet means;

the reflection element being located so that its sample contact surface provides an upper wall of the sample cuvette means.

12. An apparatus according to claim 10 or 11, characterized in that it further comprises a light source means and a light detector means.

13. An apparatus according to claim 10 or 11, characterized in that it comprises light inlet means for optically coupling the apparatus to external light source means;

light exit means for optically coupling the apparatus to external light detector means.

14. An apparatus according to claim 12 or 13 characterised in that the light source means and light detector means are capable of emitting and detecting, respectively, ultra violet and/or visible light.

15. An apparatus according to any of claims 10 to 14 characterised in that it comprises a further measuring means exposed to the sample.

16. An apparatus according to claim 15, characterised in that the further measuring means comprises an electrochemical measuring electrode.

17. A device for determining the level of an analyte in a sample of whole blood accommodated in sample container means, said device comprising,

a shield enclosing a substantial part of the device but leaving exposed a sample contact surface of an internal reflection element;

light guide means for directing light into and away from the reflection element;

means for attaching the device to the sample container means in order to maintain the device quiet relative to the sample during a measurement period and for orienting the sample contact surface of the reflection element so that the blood cells under the influence of the gravity field or a superimposed force field be removed from the sample contact surface.

18. An analyzer for the determination of the level of potassium in a sample of whole blood and comprising potassium measuring means; said analyzer being characterized by comprising means for detecting the level of hemolysis in the said sample.

19. An analyzer according to claim 18, characterized in that the means for detecting the level of hemolysis in the said sample comprises a reflection element.

20. An analyzer according to claim 18 or 19, characterized by comprising, sample inlet means;

sample cuvette means connected to the sample inlet means for accomodating the sample and exposing the sample to the reflection element, the reflection element being provided in an upper wall of the sample cuvette means.

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Fig. 1.

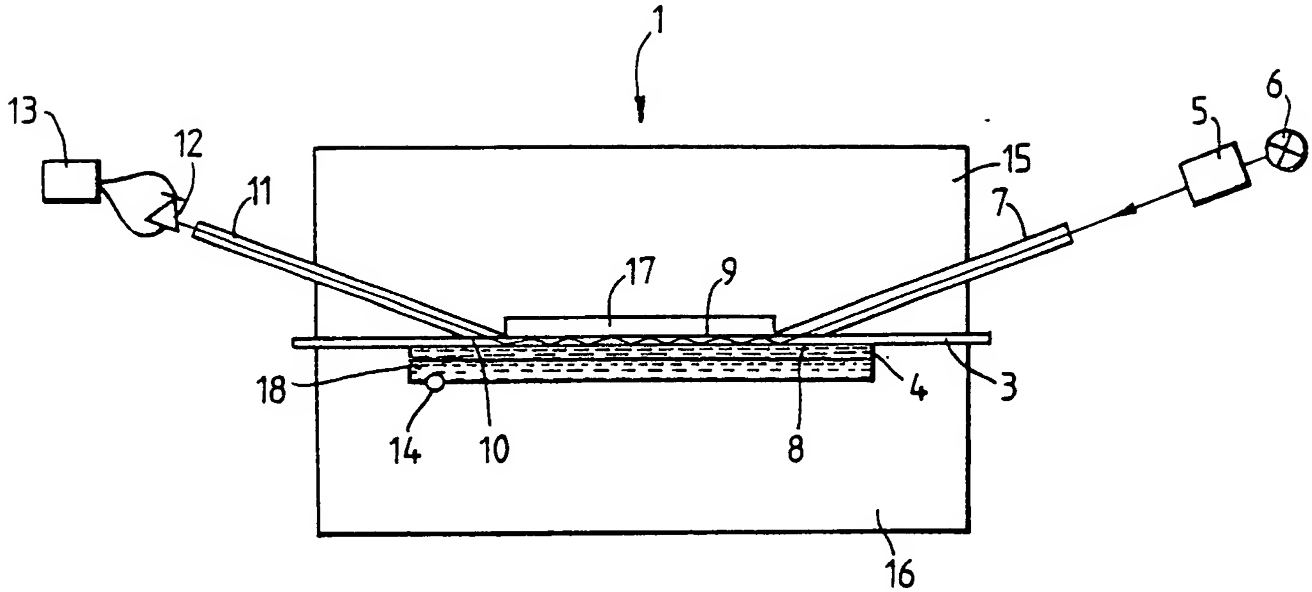

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Fig. 2.

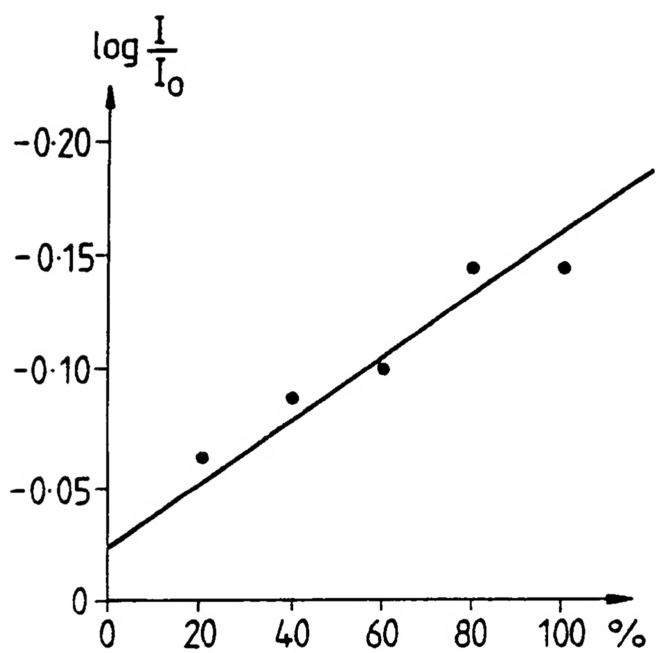

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Fig. 3.

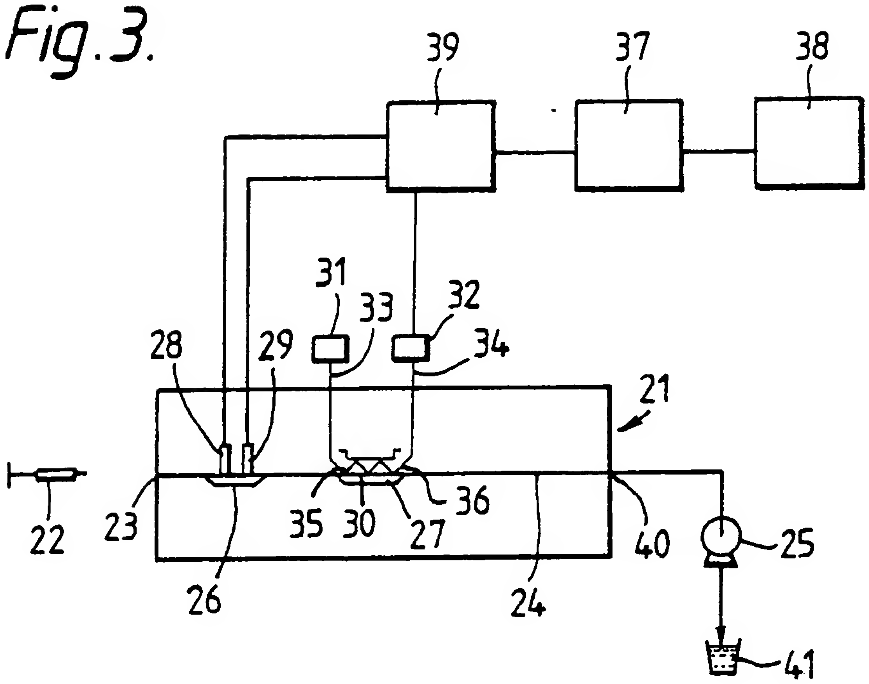
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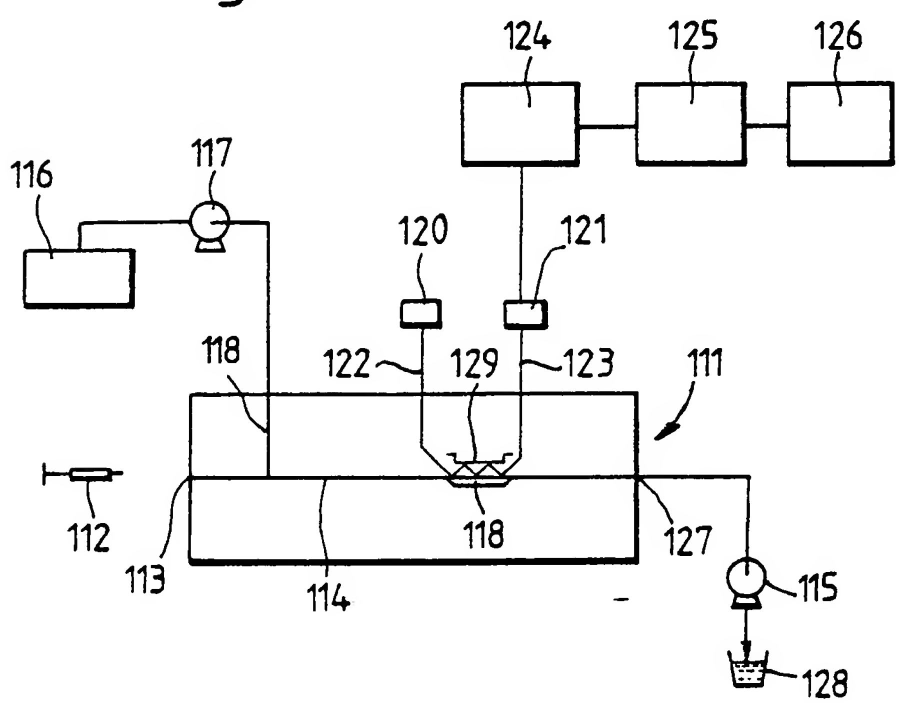
Fig. 4.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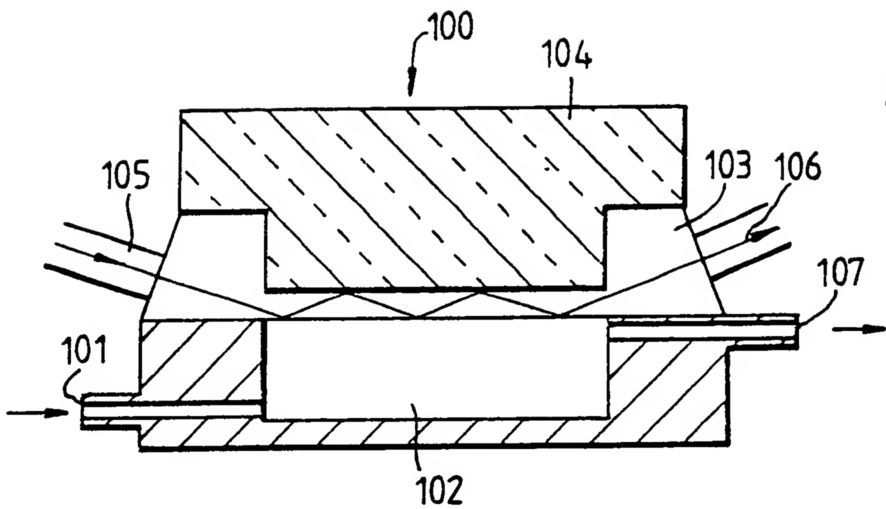


Fig.5.

Fig.6.

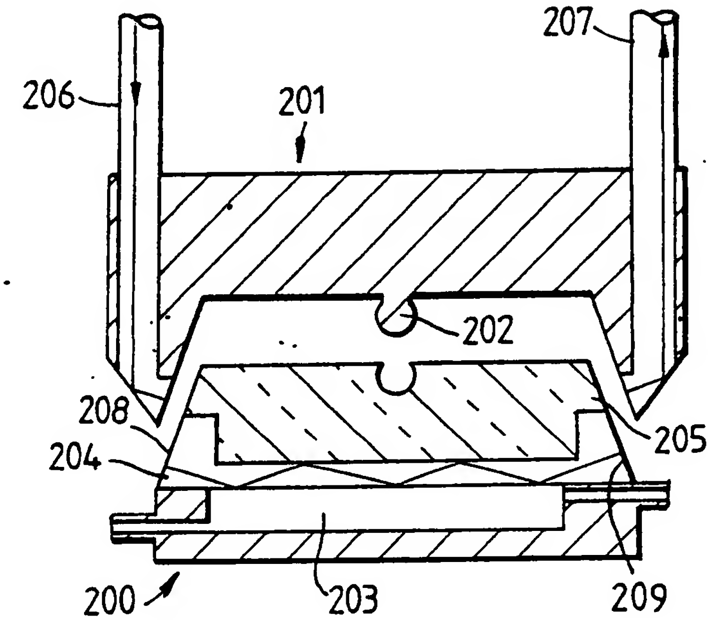


Fig. 7.

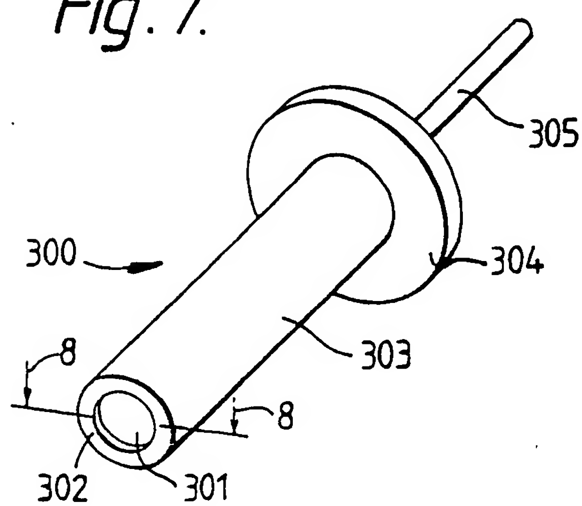
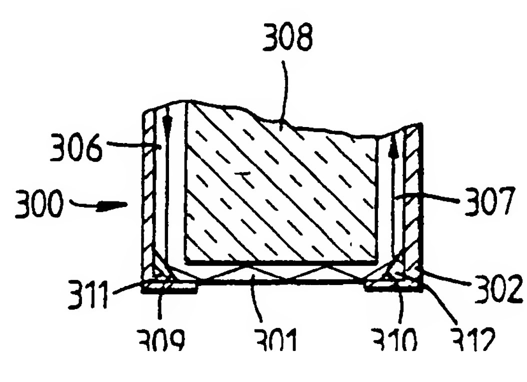


Fig.8.



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 87/00573

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^4: G 01 N 21/55

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^4	G 01 N 21/55 G 01 N 21/77 G 01 N 33/48

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	Applied Spectroscopy, volume 35, no. 4, August 1981, (New York, US), R.M. Gendreau et al.: "Fourier transform infrared spectroscopy of protein adsorption from whole blood: Ex vivo dog studies", pages 353-357 see pages 354-355; figure 2 --	1,9
A	IEEE Transactions on Biomedical Engineering, volume BME-26, no. 10, October 1979, IEEE, (New York, US), N. Kaiser: "Laser absorption spectroscopy with an ATR prism", pages 597-606 see pages 597-598 --	1,6,8
A	EP, A, 0185126 (BATTELLE MEMORIAL INSTITUTE) 25 June 1986 see page 3, lines 16-37; page 4, lines 1-7; page 8, lines 16-37; page 9, lines 1-25 --------	2,3,8,9, 11-13

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13th November 1987	11 DEC 1987
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

INTERNATIONAL APPLICATION NO. PCT/GB 87/00573 (SA 18285)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 20/11/87

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publicatio date
EP-A- 0185126	25/06/86	JP-A- 61184442	18/08/86

For more details about this annex :
see Official Journal of the European Patent Office, No. 12/82